# RES-240

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# Functional characterization of a novel class A carbapenemase CAE-1 in carbapenem-resistant *Pseudomonas aeruginosa* clinical isolates

Pseudomonas aeruginosa causes common, life-threatening infections and is exhibiting rising resistance to carbapenem antibiotics. Carbapenem-resistant P. aeruginosa (CRPA) has become a global threat due to a high mortality rate of 20–30%. An important mechanism for the development of CRPA is the presence of carbapenemases, which can hydrolyze many β-lactams.

Two carbapenem-resistant Pseudomonas aeruginosa (CRPA) isolates were collected which lacked known carbapenemases, but produced a β-lactamase CAE-1. CAE-1 was recently found in Comamonas aquatica and conferred resistance to penicillins and cephalosporins. In this study, we purpose to know whether CAE-1 was responsible for the carbapenem resistance in the CRPA isolates and its enzyme hydrolyzing characteristics.

The blaCAE-1 and blaKPC-2 were cloned and expressed in P. aeruginosa PAO1 and Escherichia coli DH5α, respectively, to test the resistance phenotypes.Both CRPA clinical isolates exhibited minimal inhibitory concentrations (MICs) of 8 μg/mL for imipenem and meropenem, with a positive result in the modified Hodge test for meropenem. Expression of blaCAE-1 in P. aeruginosa PAO1 resulted in resistance to piperacillin, ceftazidime, cefepime, and aztreonam with MICs 64 to 256 μg/mL, and intermediate to meropenem (4 μg/mL), exhibiting a broader resistance spectrum than that in E. coli DH5α.(Table 1).

| Antibiotic | MICs (μg/mL) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *P. aeruginosa* (PA56381) | *P. aeruginosa* (PA56391) | *E. coli* DH5α (pUCP24) | *E. coli* DH5α (pUCP24-$bla_{CAE-1}$) | *E. coli* DH5α (pUCP24-$bla_{KPC-2}$) | *P. aeruginosa* PAO1 (pUCP24) | *P. aeruginosa* PAO1 (pUCP24-$bla_{CAE-1}$) | *P. aeruginosa* PAO1 (pUCP24-$bla_{KPC-2}$) |
| Piperacillin | 64 | 64 | <4 | 128 | 256 | <4 | 256 | 512 |
| Piperacillin-tazobactam | 16/4 | 16/4 | <2/4 | 4/4 | 128/4 | <2/4 | 128/4 | >256/4 |
| Ticarcillin-clavulanate | 256/2 | >512/2 | <4/2 | <4/2 | 256/2 | 8/2 | >512/2 | >512/2 |
| Ceftriaxone | NA | NA | <0.25 | 16 | 8 | NA | NA | NA |
| Ceftazidime | 16 | 16 | <0.125 | 0.25 | 4 | 1 | 64 | 64 |
| Ceftazidime-avibactam | 1/4 | 1/4 | 0.06/4 | 0.125/4 | 0.06/4 | 1/4 | 1/4 | 2/4 |
| Cefepime | 16 | 16 | <0.06 | <0.06 | 2 | 1 | 128 | >128 |
| Cefoperazone-sulbactam | 16 | 16 | <0.25 | 2 | 8 | 2 | 128 | 256 |
| Aztreonam | 16 | 16 | <0.25 | <0.25 | 32 | 2 | 128 | 512 |
| Imipenem | 8 | 8 | <0.06 | 0.25 | 4 | 1 | 1 | 64 |
| Meropenem | 8 | 8 | <0.016 | 0.03 | 1 | 0.5 | 4 | >32 |
| Ertapenem | 32 | 32 | <0.03 | <0.03 | 1 | 4 | 8 | >64 |
| Levofloxacin | 0.25 | 0.5 | <0.125 | <0.125 | <0.125 | 0.25 | 0.25 | 0.25 |
| Amikacin | 4 | 4 | <1 | <1 | <1 | 2 | 2 | 2 |
| Polymyxin B | 1 | 1 | 0.25 | 0.5 | 0.5 | 1 | 1 | 1 |

Note: NA, not applicable.

Table 1 In-vitro antimicrobial susceptibility for clinical isolates and recombinant strains producing CAE-1 and KPC-2

Kinetic studies showed that CAE-1 had catalytic efficiency against all β-lactams tested, with comparatively lower efficiency against three carbapenems relative to KPC-2, while demonstrating approximately equivalent efficiency for the other β-lactam antibiotics tested(Table 2).

| Antibiotic | CAE-1 | | | KPC-2 | | |
|---|---|---|---|---|---|---|
| | *Km* (μM)[a] | *kcat* ($s^{-1}$)[a] | *kcat*/*Km*($\mu M^{-1}s^{-1}$) | *Km* (μM)[a] | *kcat* ($s^{-1}$)[a] | *kcat*/*Km*($\mu M^{-1}s^{-1}$) |
| Meropenem | 23.53±3.74 | 0.35±0.02 | 0.02 | 7.91±1.78 | 4.90±1.16 | 0.66 |
| Imipenem | 30.52±9.34 | 0.23±0.05 | 0.01 | 63.05±7.39 | 80.51±10.36 | 1.28 |
| Ertapenem | 24.27±3.82 | 1.71±0.67 | 0.07 | 22.07±6.35 | 11.85±2.00 | 0.56 |
| Piperacillin | 167.38±3.56 | 95.29±15.36 | 0.57 | 21.26±4.61 | 13.51±2.39 | 0.64 |
| Ceftazidime | 138.72±6.63 | 3.56±0.38 | 0.03 | 61.15±21.75 | 0.69±0.19 | 0.01 |
| Cefepime | 104.83±10.08 | 20.07±1.24 | 0.19 | 173.53±18.23 | 17.35±1.57 | 0.10 |
| Aztreonam | 42.07±7.50 | 8.14±0.73 | 0.20 | 92.13±22.95 | 57.86±16.69 | 0.62 |

[a] *kcat* and *Km* values were calculated as the mean+SD of three independent measurements with three different enzyme purifications.

Table 2 Kinetic parameters of CAE-1 and KPC-2

Whole-genome sequencing revealed that blaCAE-1 was a class A β-lactamase and located on an integrative and conjugative element (ICE). Comparative genomics analysis indicated that blaCAE-1 and its derivatives could be horizontally transferred via plasmids and ICEs.

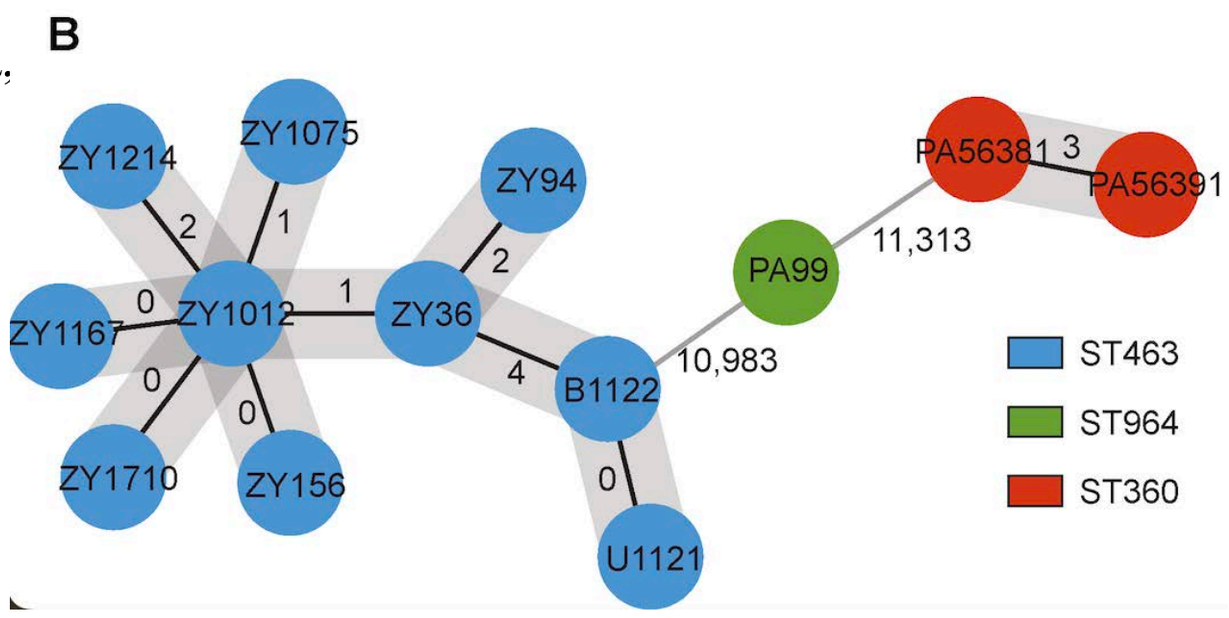


Figure 1B Minimum spanning tree based on the cgMLST analysis of 13 CAE-1 positive P. aeruginosa isolates. The node colors correspond to the sequence types to which the strains belong. Allele differences were marked adjacent to the edges.

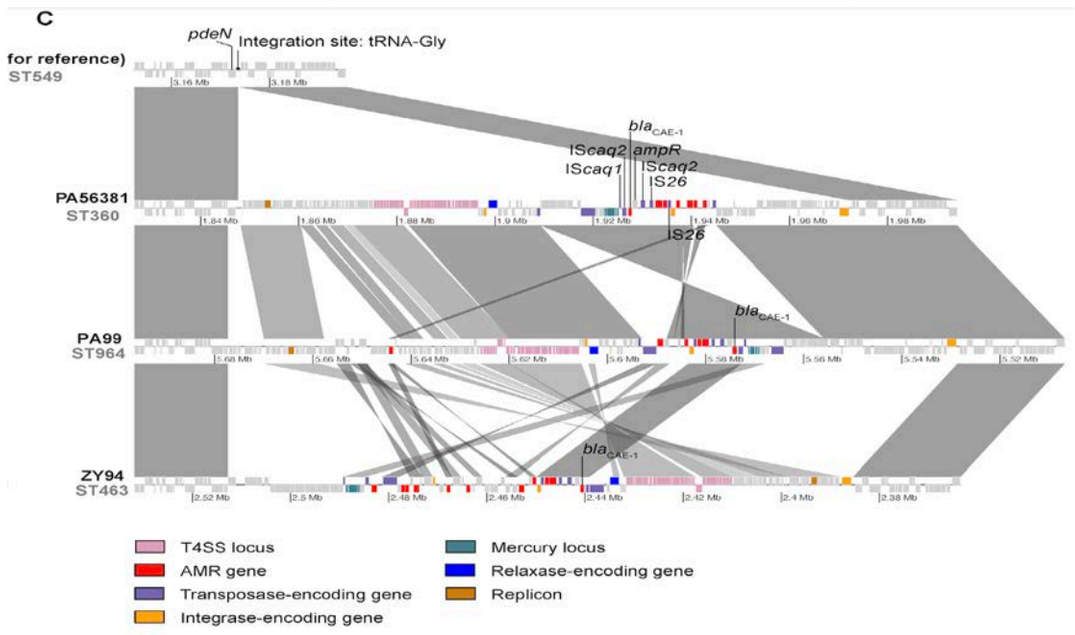


Figure 1C Comparison of the genetic context of the blaCAE-1 gene in different P. aeruginosa strains (ST360, ST964, and ST463). Strain PAO1 was used as a reference to identify the integration site of the ICE carrying blaCAE-1. Genes are represented by boxes and colored based on gene function classification. Boxes at the top of the sequence map depict genes on the forward strand, while those at the bottom depict genes on the reverse strand. Gray shadows indicate link regions that share at least 90% sequence identity.

The class A β-lactamase CAE-1 is a carbapenemase posing a high risk for horizontal dissemination. Enhanced surveillance for blaCAE-1-harboring isolates is needed.